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DATA EVALUATION RECORD

PROHEXADIONE CALCIUM

Study Type: §85-1; Metabolism of [^{14}C]BX-112 in Rats

Work Assignment No. 1-02-25 V, W, X, and Y (MRIDs 44457770-44457773)

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PROHEXADIONE CALCIUM (BX-112)

Metabolism (§85-1)

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DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat
OPPTS Number: 870.7485

OPP Guideline Number: §85-1

DP BARCODE: D246707
P.C. CODE: 112600

SUBMISSION CODE: S543930
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Prohexadione calcium (≥97.6% a.i.)

SYNONYMS: BX-112; Calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexenecarboxylate; Calcium salt of 3,5-dioxo-4-propionyl-cyclohexane-1-carboxylic acid

CITATIONS: Hallifax, D. (1993) BX-112: Absorption, Distribution, Metabolism and Excretion Study in the Rat. Pharmaco LSR Ltd., Suffolk, England, Laboratory Report No. 92/KCI113/0465, May 4, 1993. MRID 44457770. Unpublished

Hallifax, D., (1993) BX-112: Absorption, Distribution, Metabolism and Excretion Study in the Rat. Pharmaco LSR Ltd., Suffolk, England, Laboratory Report No. 92/KCI113/0465, May 28, 1993. MRID 44457771. Unpublished

Hallifax, D., (1994) BX-112: Absorption, Distribution, Metabolism and Excretion Study in the Rat. Pharmaco LSR Ltd., Suffolk, England, Laboratory Report No. 93/KCI113/0695, August 5, 1994. MRID 44457772. Unpublished

O'Connor J., (1995) BX-112: Identification of Major Metabolite in the Rat. Pharmaco LSR Ltd., Suffolk, England, Laboratory Report No. 95/KCI155/0210, June 23, 1995. MRID 44457773. Unpublished

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EXECUTIVE SUMMARY:

In a rat metabolism study (MRIDs 44457770-44457773), [^{14}C-C3 or C5-cyclohexene]BX-112 (≥97.6 % a.i.) was administered to Fischer 344 rats (5/sex/dose) as a single oral (gavage) dose at 50 or 500 mg/kg or as a single oral dose at 50 mg/kg following a 14-day pretreatment with BX-112 at 50 mg/kg. In addition, four male and female bile-cannulated Fischer 344 rats were administered a single oral dose of [^{14}C]BX-112 at 50 mg/kg.

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Within 168 hours of oral dosing with [^{14}C]BX-112 at 50 or 500 mg/kg, 91.8-109% of the dosed radioactivity was recovered from male and female rats within each dose group, except bile-cannulated rats. There were no sex-related differences in the pattern of excretion, and pretreatment had no effect on the amount or route of excretion. For low-dose animals (with or without pretreatment), renal excretion was the primary route of elimination accounting for 76.2-84.6% of the dose, with fecal excretion accounting for 17.2-24.7% of the dose. When the dose was increased to 500 mg/kg, fecal excretion (58.2-60.9% dose) became the primary route of elimination, with renal excretion decreasing to 33.6-40.1% of the dose. Excretion of radioactivity in both the bile (≤0.3% dose) and expired air (≤0.1% dose) was minor. By 168 hours post-dose, ≤0.1% of the dose remained in the carcass for each dose group.

In both the low- and high-dose groups, the concentration of radioactivity in blood increased to maximum levels by 0.5 hours post-dose and declined thereafter. In males, the absorption K_a, elimination $T_{½}$, and time to the inflexion were not affected by dose, but increasing the dose increased the distribution $T_{½}$ (1.9x) and the AUC value (2.6x). Except for an increase in the distribution $T_{½}$ (2.2x), none of the plasma kinetic parameters for females were substantially affected by the dose level. The relatively minor increases (1.2-2.6x) in plasma AUC values between low- and high-dose animals supports the observation that absorption of [^{14}C]BX-112 was limited at the high-dose level.

Concentrations of radioactivity in most tissues/organs within each dose group were similar between the sexes over time, although levels of radioactivity generally declined more gradually in tissues of females than males. Maximum concentrations of radioactivity in most tissues were attained within 0.5 hours of dosing. By 168 hours post-dose, average concentrations of radioactivity were ND-0.105 µg/g in the low-dose and ND-1.65 µg/g in the high dose group. There was no evidence of accumulation in specific organs or tissues.

The relative distribution of radioactivity among tissues was similar between dose levels and sexes. Excluding the G.I. tract, maximum concentrations of radioactivity attained in tissues/organs were highest in the lymph nodes (142-445 µg/g), kidneys (96.8-191 µg/g), pancreas (54.2-179 µg/g), spleen (33.9-91.4 µg/g), and liver (29.9-80.6 µg/g). Relatively high levels of radioactivity were also observed in the uterus (76.7-173 µg/g) and ovaries (83.6-83.8 µg/g) of females. The lowest concentrations of radioactivity were initially (0.5 hour) observed in bone (2.87-6.36 µg/g). Increasing the dose level increased the concentration of radioactivity in tissues, but not in proportion to the 10x increase in dose level. Maximum concentrations of radioactivity in the above tissues were 1-3.3x higher in high-dose animals than in low-dose animals. Repeated dosing at 50 mg/kg for 14 days prior to dosing with [^{14}C]BX-112 at 50 mg/kg had no effect on the accumulation of radioactivity in tissues/organs.

Analyses of urine and fecal extracts identified and/or characterized 55.8.4-75.3% of the dosed radioactivity for each dose group. Metabolism of BX-112 was qualitatively and quantitatively similar between sexes, although there were minor quantitative differences between males and females in the high-dose and repeated low-dose groups. Metabolism was also qualitatively similar between dose groups.

In the low-dose groups (with or without pretreatment), the major metabolite in excreta was the free acid metabolite, KI-2817 (38.3-53.7% dose), which was excreted primarily in the urine (20.2-31.6% dose). The only other significant component isolated from excreta of low-dose rats was the putative base-labile conjugate of KI-2817, which accounted for 15.5-21.3% of the dose in urine. In the high-dose group, KI-2817 (60.9-68.0% dose) was also the major metabolite in excreta. However, the majority of KI-2817 (53.4-64.6% dose) from high-dose rats was recovered in the feces rather than in urine, and levels of both KI-2817 and its putative base-labile conjugate were lower (3.4-7.5% dose) in urine. Minor amounts of KI-5376 (1.1-2.3% dose) were also identified in the feces of high-dose rats. KI-2817 was also identified as the principal metabolite (28.5-76.6% of tissue radioactivity) in liver and kidney extracts from low and high-dose males.

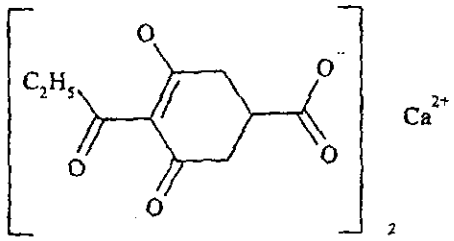
This study is classified **acceptable (§85-1)** and satisfies the requirement for a metabolism study in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. Materials

1. Test compounds: Prohexadione calcium; BX-112
   Purity: 93.8% a.i.
   Batches: G14-10 and G14-12
   Description: Fine white or cream colored powder
   Contaminants: Not specified
   Storage: Refrigerated at 4°C in the dark
   CAS #: 127277-53-6
   Structure:



   [^{14}C-C3 or C5-cyclohexene]BX-112
   Radiochemical purity: ≥97.6% (by TLC and HPLC)
   Specific activity: 3.6M Bq/mg; 97.8 µCi/mg
   Batch No.: CP-1107

2. Vehicle: Suspension of 0.5% methoxy-cellulose in distilled water.

3. Test animals: Species: Rat
   Strain: Fischer 344
   Age: 5-6 weeks old
   Weight at study initiation: Males, 88-173 g; females, 78-129 g
   Source: Charles River (UK) Ltd., Margate, Kent
   Housing: During acclimation, rats were housed in groups of 5 or less in stainless steel cages; for the mass balance and biliary excretion studies, rats were housed individually in all-glass metabolism cages suitable for the separate collection of urine and feces; and for the blood kinetic and tissue distribution studies, rats were housed individually in Type RB3 G cages with polypropylene bodies and stainless steel mesh lids and floors.
   Acclimation period: Not specified.
   Diet: Laboratory Animal Diet No. 1 (LAD-1) pelleted diet, Special Diets Services, Witham, Essex, England, ad libitum; animals were fasted overnight prior to ^{14}C-dosing and for 4 hours post-^{14}C-dose.

Water: Source not specified, ad libitum
Environmental conditions:
Temperature: 21 ± 3°C
Humidity: 55 ± 15%
Air Changes: Not specified
Photoperiod: Not specified

4. Observations: Animals were inspected twice daily for evidence of treatment-related effects or illness.

5. Preparation of dosing solutions: Dosing solutions were prepared by isotopically diluting [^{14}C]BX-112 with non-radiolabeled BX-112 and then dissolving the diluted [^{14}C]test substance in aqueous 0.5% methyl-cellulose. Each formulation was prepared immediately prior to dosing, and quality control samples were taken to determine the final specific activity and homogeneity of each dosing solution. The final specific activity of each dosing solution was 2.85-6.29 dpm/ng for the low-dose groups and 0.402-0.558 dpm/ng for the high-dose groups.

B. Study Design

These studies were designed to determine the absorption, metabolism, distribution, and excretion of [^{14}C]BX-112 as a function of single and repeated oral dosing of rats. In a preliminary study examining the recovery of radioactivity in expired air, a single male Fischer 344 rat was dosed orally by gavage with [^{14}C]BX-112 at 50 or 500 mg/kg. In the subsequent mass balance study, three groups of Fischer 344 rats (5/sex/dose group) were dosed orally (gavage) with [^{14}C]BX-112 at target doses of 50 or 500 mg/kg or 50 mg/kg following a 14-day pretreatment with non-radiolabeled BX-112 at 50 mg/kg body weight/day. Two other groups of rats (5/sex/dose) were dosed orally once with [^{14}C]BX-112 at 50 or 500 mg/kg to examine kinetics in the blood, and another two groups (20/sex/dose) were dosed orally once with [^{14}C]BX-112 at 50 or 500 mg/kg to examine distribution in tissues over time. A biliary excretion study was also conducted using 4 bile-cannulated rats/sex dosed orally with [^{14}C]BX-112 at 50 mg/kg. Animals were randomly assigned to dose groups. Actual average doses for each test group are presented in Table 1 and were within 92-120% nominal of the 50 mg/kg dose and 79-131% nominal of the 500 mg/kg dose.

The study protocol stated that the low-dose level of 50 mg/kg level was chosen as it represents a no-effect level, but the selection of 500 mg/kg for the high-dose was arbitrary. An intravenous dosing group was not included in these studies as BX-112 is of limited solubility (200 ppm) in water or saline. The in-life portion of this study was conducted from July 31, 1990 to April 30, 1992.

Table 1. Dose groups for [^{14}C]BX-112 rat metabolism study.

Dose Group (Group Nos.)	Nominal dose (mg/kg)	Actual average dose (mg/kg) [a]	# animals per dose group	Comments
Single oral low or high dose (Group 1)	50/500	Low: 51.9 High: 525	1 male/ dose	Preliminary Study: Urine, feces, $^{14}CO_2$ and ^{14}C-volatiles were collected for up to 24 hours.
Single oral low dose (Group 2)	50	Male: 46.3 Female: 49.2	5/sex	Mass Balance Study: Urine, feces, and cage wash samples were collected at 6, 12, 24, 72, 96, 120, 144, and 168 hours post-dose, and organ and tissue samples were collected at sacrifice (168 hours post-dose). In addition, pooled 0-48 hour samples of urine and feces from each dose group were used for metabolite identification/characterization.
Single oral high dose (Group 3)	500	Male: 656 Female: 575	5/sex	
Repeated oral low dose [b] (Group 14)	50	Male: 52.3 Female: 59.2	5/sex	
Single oral low dose (Group 4)	50	Male: 56.2 Female: 52.0	5/sex	Blood/Plasma Kinetics Study: Whole blood was sampled at 0.08, 0.17, 0.3, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 96 hours post-dose, and subsamples were centrifuged to obtain plasma.
Single oral high dose (Group 5)	500	Male: 581 Female: 504	5/sex	
Single oral low dose (Groups 6-9)	50	Male: 51.9-55.0 Female: 53.7-59.8	5/sex/group (20/sex)	Tissue Distribution Study: 5 rats/sex/dose group were sacrificed at 0.5, 3, 6, and 96 hours post-dose, and organ and tissue samples were collected and radioassayed.
Single oral high dose (Groups 10-13)	500	Male: 490-523 Female: 395-482	5/sex/group (20/sex)	
Single oral low dose (Group 15)	50	Male: 46.1 Female: 51.0	4/sex	Biliary Excretion Study: bile was sampled hourly up to 6 hours and at 6-12 and 12-24 hours post-dose. Urine, feces, and cage washes were collected 0-6, 6-12, and 12-24 hours post-dose. At 24 hours post-dose, rats were sacrificed and the liver and G.I. tract were collected from each rat.

a Data were obtained from Appendix I, pages 113-116 of MRID 44457770; for the tissue distribution study, the range of averages from the 4 groups/sex are presented.

b The actual average daily dose of BX-112 given during the 14-day pretreatment interval was not specified.

1. Dosing and sample collection

The test animals were dosed orally by gavage at a target dose of 50 or 500 mg/kg body weight; the target volume for dosing was not specified. Animals were weighed prior to ^{14}C-dosing to determine dose per animal, and the actual dose administered was

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determined by weighing the syringe before and after dosing. In addition, the syringe and cannula were washed with 0.5% phosphoric acid to determine residual dose. Animals were fasted for 16 hours prior to dosing with [^{14}C]BX-112 and for 4 hours post-dose.

In the preliminary study, urine, cage washes, feces, and expired air were collected separately for the 0-6 and 6-24 hour intervals after dosing. ^{14}C-Volatile organics and $^{14}CO_2$ were collected in a series of four traps; the first two traps contained 2M NaOH for trapping $^{14}CO_2$ and the next two traps contained 2-ethoxyethanol for trapping other ^{14}C-volatiles. At each sampling interval, cages were washed with 0.5% phosphoric acid.

In the mass balance study (Groups 2, 3, and 14), urine and feces were collected separately over ice from each animal at 0-6, 6-12, 12-24 hour intervals and at 24-hour intervals thereafter up to 168 hours post-dose. At each sampling interval, a separate cage wash sample was also obtained. At 168 hours post-dose, animals were sacrificed by an overdose of anesthetic and the following organ/tissue samples were collected:

adrenals	small intestines (plus contents)	residual carcass
blood	kidneys	salivary glands
bone (femur)	liver	skin
brain	lung	spleen
caecum (plus contents)	lymph nodes (mesenteric)	stomach (plus contents)
eyes	muscle (skeletal)	submaxillary gland
fat (abdominal)	ovaries	testes
harderian glands	pancreas	thymus
heart	plasma	thyroid
large intestines (plus contents)		uterus

For the blood kinetics study (Groups 4 and 5), 0.2-0.3 mL of blood were withdrawn from each rat at 0.08, 0.17, 0.33, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 96 hours after dosing, and subsamples of blood were centrifuged to obtain plasma.

For the tissue distribution study (Groups 6-13), 5 rats/sex/dose were sacrificed at 0.5, 3, 6, and 96 hours post-dose and the same tissues/organs were sampled as are listed above for the mass balance study groups.

In the biliary excretion study, 4 rats/sex (Group 15) were anesthetized with halothane and their bile ducts were cannulated. After recovery and dosing with [^{14}C]BX-112 at 50 mg/kg, bile was collected hourly up to 6 hours post-dose and then from 6-12 and 12-24 hours post-dose. Urine, feces, and cage washes were collected 0-6, 6-12, and 12-24 hours post-dose. Animals were sacrificed at 24 hours post-dose and the liver and gastro-intestinal tract of each animal was collected and divided into the caecum and large and small intestines. Blood samples were also collected from all four males, but from only one female due to the death of two bile-cannulated females and poor health of another female.

Samples of tissues, blood, cage wash, and excreta were stored at -20°C until analysis.

2. Radioassay

Samples of urine, cage washes, plasma, bile, and trapping solutions were analyzed for total radioactivity directly by liquid scintillation counting (LSC). Feces were homogenized with 0.5% phosphoric acid (1:2, v/v), combusted, and then radioassayed by LSC. Samples of whole blood, tissues, organs, and carcass were homogenized in water (1:1, v/v) if necessary and solubilized prior to LSC. The reported limit of detection for the radioassay was ~0.002 µg/g for samples from low-dose animals and ~0.02 µg/g for samples from high-dose animals.

3. Metabolite characterization

a. Excreta

Urine and fecal samples collected 0-48 hours post-dose from animals in the mass balance study (Groups 2, 3, and 14) were used for characterization and quantitation of metabolites in excreta. Approximately half of each urine sample and fecal homogenate sample was pooled by sex/dose group. Two sets of analyses were conducted on pooled urine and fecal homogenates.

In the initial set of analyses, acidified (pH 3) urine samples were extracted using a C_2-Solid Phase Extraction (SPE) column preconditioned with methanol and water (pH 3). After eluting through the urine sample, the SPE column was eluted with acetone yielding an organic fraction that was concentrated under N_2. Recovery of radioactivity from the C_2-SPE column was 77.3-78.9% for the low-dose group, 73.5-75.3% for the high-dose group, and 86.8-92.1% for the repeated low-dose group. The resulting urinary extracts were analyzed by reverse-phase (RP) HPLC using a C_8 column.

In the second set of analyses, acidified (pH 3) urine samples were extracted using a C_{18}-SPE column preconditioned with methanol and water (pH 3). After eluting through the urine sample, the SPE column was eluted with acetone, as in the first analyses, yielding an organic fraction that was concentrated under N_2. The C_{18} column was then eluted sequentially with water (pH 3) and 1 M NaOH. The acidified water eluant was combined with the initial urine eluant, and the NaOH eluant and column stationary phase were radioassayed. The total recovery of urinary radioactivity in the organic and aqueous eluants from the C_{18}-SPE column was 88.6-90.7% for the low-dose group, 89.7-103.1% for the high-dose group, and 92.7-96.9% for the repeated low-dose group. Both the organic and combined aqueous eluants were analyzed by RP-HPLC using a C_{18} column.

In addition, urine pooled from each dose group was also subjected to acid/base and enzymatic hydrolyses. One subsample was base hydrolyzed in 1M NaOH for 6 hours

at an unspecified temperature, and other subsamples were incubated with β-glucuronidase (with and without sulfatase activity) in 0.05 M acetate buffer (pH 5.0) at 37°C for 22 hours. Samples were also acid hydrolyzed with 0.2 N HCl at room temperature for 5 minutes, conditions under which glucuronide ester conjugates are expected to be stable. The treated urine subsamples were extracted using the C_2-SPE column as described above, and the resulting eluants were analyzed by RP-HPLC.

Pooled fecal homogenates were acidified with 1M sulfuric acid, diluted with acetone, shaken for 0.5 hours, and centrifuged. The resulting supernatants were concentrated under N_2. The residual fecal solids were radioassayed. Recovery of radioactivity in the fecal extracts was 83.4-84.4% for the low-dose group, 92.3-92.4% for the high-dose group, and 83.3-84.8% for the repeated low-dose group. Fecal extracts were analyzed by RP-HPLC.

Urinary and fecal extracts were initially analyzed by RP-HPLC using a C_8 column and a mobile phase gradient of water to acetonitrile (each containing 0.2% trifluoroacetic acid, TFA), with UV (274 nm) detection and ^{14}C-detection. However, due to the lower recoveries of radioactivity from the initial C_2-SPE extractions of urine and the appearance of artifacts in the initial HPLC analyses using the C_8 column, the urine samples were reextracted using the C_{18} SPE column described above and both urinary and fecal extracts were reanalyzed using the same HPLC system with a C_{18} column. Results from the definitive analyses using the C_{18} column are reported in MRID 44457771. Although artifacts were present in the initial HPLC analyses using the C_8 column, results from the C_8 analyses of urine were similar to the C_{18} analyses, with regards to the major metabolites, and are reported in MRID 44457772. For purposes of this report, only the definitive analyses using the C_{18} column are discussed.

The initial identification and characterization of selected urinary metabolites was conducted by comparison of HPLC retention times to six reference standards and by LC/MS analysis. Pooled urine samples from high-dose males were also used for further analyses (MRID 44457773) of the major unknown urinary metabolite. The pooled samples were extracted as described above using a C_{18} SPE column and analyzed by HPLC, FAB-MS, and Atmospheric Pressure Electrospray HPLC/MS.

b. Liver and Kidney

Samples of liver and kidney from a single low-dose and high-dose male sacrificed 0.5 hours after dosing (Groups 6 and 10) were used to characterize ^{14}C-residues in these tissues. These liver and kidney samples respectively accounted for 3.2 and 2.7% of the dosed radioactivity in the low-dose male, and 0.7 and 0.4% of the dosed radioactivity in the high-dose male.

Liver and kidney homogenates were each acidified with 1M sulfuric acid, extracted twice with acetone by shaking for 0.5 hours, and centrifuged. The supernatants were combined, concentrated, and lyophilized, and the resulting residue was resuspended in 0.2% aqueous TFA and centrifuged. Extracts and residual tissue solids were radioassayed. The majority of radioactivity (83.4%) was extracted from kidneys of both low- and high-dose males, and 35.1% and 59.5% of the radioactivity in liver was extracted from liver of low- and high-dose males, respectively. Liver and kidney extracts were analyzed by RP-HPLC.

3. Statistics

Radioactivity in excreta, tissues, organs, and blood were reported as the % administered dose both for individual samples and as the mean (±S.D.) of 4 or 5 animals/sex/dose group. Concentration of radioactivity in tissues/organs, whole blood and plasma were also reported as ng equivalents of BX-112/g sample weight. Pharmacokinetic analyses of radioactivity in blood and plasma were conducted using a non-linear, least-squares regression (PROC NLIN procedures; SAS Institute Inc., Cary, NC) and a two-compartmental model with an absorption phase. The first compartment was assumed to represent a phase in which distribution of radioactivity predominates and the second compartment was assumed to represent a phase in which elimination predominates. The model is described by the following equation:

$$C_{(t)} + Ae^{-\alpha t} + Be^{-\beta t} - C_{(0)}e^{-ka\,t}$$

where: $C_{(t)}$ is the concentration at time t;
A and B are intercepts on the concentration axis (ng eq/ml);
α and β are the slopes (h^{-1}) of the curves for the two compartments;
$C_{(0)}$ is the concentration at time zero (ng eq/ml); and
ka is the rate constant (h^{-1}) for the absorption phase.

The half-life ($T_{½}$) for each phase was calculated as follows:

$$T_{½} \text{ in hours} = 0.693/\text{rate constant}$$

Areas under the concentration:time curves were calculated as follows:

$$AUC \text{ (ng eq/mL } h^{-1}) = A/\alpha + B/\beta - C_{(0)}/ka$$

II. RESULTS

A. Preliminary Study

Within 24 hours of administering a single oral dose of [^{14}C]BX-112 at 50 or 500 mg/kg, 84.2 and 89.8% of the dose was recovered in excreta and expired air of a single male rat per dose. For the low-dose male, the majority of the dosed radioactivity was recovered in the urine plus cage washes (68.6% dose), while 15.5% of the dose was recovered in the feces. In contrast, the majority of the radioactivity for the high-dose male was recovered in the feces (77.5% dose), whereas the urine plus the cage washes accounted for 12.2% of the dose. At both levels, <0.05% of the dose was recovered as expired organic volatiles and ≤0.1% of the dose was recovered as expired $^{14}CO_2$.

Table 2. Recovery of radioactivity in expired air and excreta of rats 24 hours after dosing with [^{14}C]BX-112 at 50 or 500 mg/kg.[a]

Sample	Percent of radioactive dose administered					
	Low dose			High dose		
	0-6 hr	6-24 hr	Total	0-6 hr	6-24 hr	Total
^{14}C-organic volatiles	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
$^{14}CO_2$	0.1	<0.05	0.1	0.1	<0.05	0.1
Urine	34.5	2.8	37.3	6.9	0.6	7.5
Cage Washes	27.6	3.7	31.3	4.2	0.5	4.7
Feces	4.5	11.0	15.5	31.6	45.9	77.5
Total	66.7	17.5	84.2	42.8	47.0	89.8

[a] Data were from one male rat/dose and were obtained from Table 1, page 90 of MRID 44457770.

B. Clinical Observations - Main studies

1. Toxicity and mortality - There were no signs of toxicity in any animals following dosing at either 50 or 500 mg/kg. However, one of the low-dose males from the blood kinetics study was reported to have died 6 hours after dosing. No explanation of the death was provided.

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C. Main Study - Pharmacokinetics

1. Absorption

Absorption of [^{14}C]BX-112 from the G.I. tract of rats was evident in low- and high-dose animals based upon the urinary excretion of radioactivity. Within 6 hours of dosing with [^{14}C]BX-112 at 50 mg/kg, 57.7-73.7% of the dosed radioactivity was excreted in the urine (plus cage wash), and cumulative renal excretion accounted for 76.2-84.6% of the dose within 168 hours. There were no sex-related differences in the overall absorption of radioactivity for both low- and high-dose animals, and pretreatment had no effect on absorption. However, absorption was limited at the 500 mg/kg dose level as total renal excretion was reduced to 33.6-40.1% of the dose and data from bile cannulated rats indicated that ≤0.3% of the dose was excreted in the bile within 24 hours of dosing.

a) Single low dose: Within 6 hours of oral dosing with [^{14}C]BX-112 at 50 mg/kg, radioactivity recovered in the urine (plus cage wash) accounted for 67.0% of the dose for males and 57.7% of the dose for females (Table 3). By 24 hours post-dose, cumulative urinary excretion was 78.8 and 72.2% of the dose for males and females, respectively, and fecal excretion accounted for 18.2 and 21.4% of the dose for males and females.

Table 3. Recovery over time of radioactivity in excreta of rats dosed orally (gavage) with [^{14}C]BX-112 at 50 mg/kg.[a]

Sample	Percent of radioactive dose administered								
	Males								
	6 hr	12 hr	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr
Urine	49.5	5.3	1.5	1.1	0.3	0.2	0.2	0.1	0.1
Cage wash	17.5	3.7	1.3	0.5	0.4	0.3	0.2	0.1	0.3
Feces	5.4[b]	10.6	2.2	0.6	0.2	0.2	0.1	0.1	<0.05
Total	72.4	19.6	5.0	2.2	0.9	0.7	0.5	0.3	0.4
Sample	Females								
	6 hr	12 hr	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr
Urine	45.2	5.6	1.9	1.1	0.2	0.1	0.1	0.1	0.1
Cage wash	12.5	5.4	1.6	0.8	0.5	0.2	0.2	0.1	0.3
Feces	5.1[c]	11.4	4.9	1.1	0.4	0.1	<0.05	<0.05	<0.05
Total	62.8	22.4	8.4	3.0	1.1	0.4	0.3	0.2	0.4

a Data are the mean of five animals/sex at each sampling interval, unless otherwise indicated, and were obtained from Table 2, page 91 of MRID 44457770.

b Data are the average of three males; two males produced no feces during this interval.

c Data are the average of two females; three females produced no feces during this interval.

b) Single high dose: Increasing the oral dose to 500 mg/kg, decreased the relative absorption of [^{14}C]BX-112. Within 6 hours of dosing with [^{14}C]BX-112 at 500 mg/kg, radioactivity recovered in the urine (plus cage wash) accounted for 19.9% of the dose for males and 12.1% of the dose for females (Table 4). By 24 hours post-dose, cumulative urinary excretion was 38.3 and 32.7% of the dose for males and females, respectively, and fecal excretion accounted for 59.9 and 69.1% of the dose for males and females.

Table 4. Recovery over time of radioactivity in excreta of rats following administration of a single oral (gavage) dose of [^{14}C]BX-112 at 500 mg/kg.[a]

Sample	Percent of radioactive dose administered								
	Males								
	6 hr	12 hr	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr
Urine	10.0	4.6	0.7	0.3	0.1	0.1	0.1	<0.05	<0.05
Cage wash	9.9	10.8	2.3	0.2	0.1	<0.05	0.6	<0.05	<0.05
Feces	0.1 [b]	28.8	31.0	0.9	0.1	<0.05	<0.05	<0.05	<0.05
Total	20.0	44.2	34.0	1.4	0.3	0.1	0.7	<0.05	<0.05
Sample	Females								
	6 hr	12 hr	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr
Urine	6.5	1.3	1.3	0.2	0.1	<0.05	<0.05	<0.05	<0.05
Cage wash	5.6	15.8	2.2	0.3	0.1	<0.05	<0.05	<0.05	<0.05
Feces	1.2 [c]	38.1 [d]	29.8	4.6	0.4	<0.05	<0.05	<0.05	<0.05
Total	13.3	55.2	33.3	5.1	0.6	<0.05	<0.05	<0.05	<0.05

a Data are the mean of five animals/sex at each sampling interval, unless otherwise indicated, and were obtained from Table 3, page 92 of MRID 44457770.
b Data are from one male; four males produced no feces during this interval.
c Data are the average of two females; three females produced no feces during this interval.
d Data are the average of three females; two females produced no feces during this interval.

c) Repeated low dose: Pretreatment had little effect upon the rate of absorption as the relative levels of radioactivity excreted in the urine and feces were similar to the single low dose group. Within 6 hours of oral dosing with [^{14}C]BX-112 at 50 mg/kg following 14 days of treatment with BX-112 at 50 mg/kg, radioactivity recovered in the urine (plus cage wash) accounted for 66.5% of the dose for males and 73.7% of the dose for females (Table 5). By 24 hours post-^{14}C-dose, cumulative urinary excretion was 76.5 and 82.2% of the dose for males and females, respectively, and fecal excretion accounted for 24.5 and 27.0% of the dose for males and females.

Table 5. Recovery over time of radioactivity in excreta of rats dosed orally (gavage) with [^{14}C]BX-112 at 50 mg/kg following 14 days of dosing with BX-112 at 50 mg/kg.[a]

Sample	Percent of radioactive dose administered								
	Males								
	6 hr	12 hr	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr
Urine	57.3	3.0	2.4	0.9	0.3	0.1	0.2	0.1	<0.05
Cage wash	9.2	3.9	0.7	0.3	0.2	0.2	0.1	0.1	0.1
Feces	6.9[b]	4.6[c]	13.0	3.6	0.3	0.1	0.2	0.1	<0.05
Total	73.4	11.5	16.1	4.8	0.8	0.4	0.5	0.3	0.1
Sample	Females								
	6 hr	12 hr	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr
Urine	43.0	3.9	1.6	0.7	0.3	0.2	0.2	0.1	0.1
Cage wash	30.7	2.1	0.9	0.4	0.1	0.1	0.1	0.1	0.1
Feces	NS[d]	11.2[e]	15.8	1.7	0.2	0.1	0.1	<0.05	<0.05
Total	73.7	17.2	18.3	2.8	0.6	0.4	0.4	0.2	0.2

a Data are the mean of five animals/sex at each sampling interval unless otherwise specified, and were obtained from Table 20, page 109 of MRID 44457770.
b Data are the average of two males; three males produced no feces during this interval.
c Data are the average of four males; one male produced no feces during this interval.
d NS = no samples.
e Data are the average of three females; two females produced no feces during this interval.

d) Bile-canulated rats - single low dose: Absorption and biliary excretion was not a major route of elimination for [^{14}C]BX-112 as ≤0.3% of the dose radioactivity was recovered in the bile of males and females within 24 hours of oral dosing with [^{14}C]BX-112 at 50 mg/kg. Levels of renal excretion in bile-cannulated rats were similar to the single low-dose group. Within 6 hours of oral dosing with [^{14}C]BX-112 at 50 mg/kg, radioactivity recovered in the urine (plus cage wash) accounted for 70.3% of the dose for males and 58.5% of the dose for females (Table 6). By 24 hours post-dose, cumulative urinary excretion was 84.0 and 76.1% of the dose for males and females, respectively. However, for the same 0-24 hour interval, fecal excretion of radioactivity was lower in bile-cannulated rats (6.6-10.7% dose) than in the single low-dose group (18.2-21.4% dose).

Table 6. Recovery over time of radioactivity in bile and excreta of rats dosed orally (gavage) with [^{14}C]BX-112 at 50 mg/kg.[a]

Sample	Percent of radioactive dose administered							
	Males							
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	12 hr	24 hr
Bile	0.1	0.1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Urine	NS [b]	NS	NS	NS	NS	49.1	6.0	2.7
Cage wash	NS	NS	NS	NS	NS	21.2	2.4	2.6
Feces	NS	NS	NS	NS	NS	2.3	3.8	4.6
Total	0.1	0.1	<0.05	<0.05	<0.05	72.6	12.2	9.9
Sample	Females							
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	12 hr	24 hr
Bile	<0.05	0.1	<0.05	<0.05	<0.05	<0.05	0.1	0.1
Urine	NS	NS	NS	NS	NS	33.7	2.6	9.1
Cage wash	NS	NS	NS	NS	NS	24.8	3.8	2.1
Feces	NS	NS	NS	NS	NS	0.7	5.2	0.7
Total	<0.05	0.1	<0.05	<0.05	<0.05	59.2	11.7	12.0

a Data are the mean of four rats/sex and were obtained from Table 22, page 111 of MRID 44457770.
b NS = no samples.

2. Blood and plasma kinetics

The average concentration of radioactivity (µg BX-112 equivalents/mL) in blood and plasma over time of male and female rats dosed with [^{14}C]BX-112 at 50 or 500 mg/kg are presented in Table 7. Data are missing from some of the rats (5/sex/dose) as one of the low-dose males died between the 4- and 6-hour sampling intervals, and blood samples were difficult to obtain from several animals at different time points, most notably for two females.

In both sexes from the low- and high-dose groups, the average concentration of radioactivity in both whole blood and plasma increased to maximum levels by 0.5 hours post-dose and declined thereafter. In the low-dose group, maximum concentrations of radioactivity in whole blood (males - 40.5 µg/mL; females - 44.3 µg/mL) and plasma (males - 75.3 µg/mL; females - 78.6 µg/mL) were similar between sexes. However, in the high-dose group, the maximum concentration of radioactivity in blood and plasma of males (blood - 61.1 µg/mL; plasma - 110 µg/mL) was approximately 2x higher than in females (blood - 34.5 µg/mL; plasma - 57.6 µg/mL).

For males, the maximum concentration of radioactivity in blood and plasma was higher (1.5x) in high-dose group than in the low-dose group, but did not reflect the 10-fold increase in dose. For the females, the maximum concentration of radioactivity in blood and plasma was actually lower (~0.8x) in high-dose group than in the low-dose group.

Table 7. Concentration of radioactivity (µg equivalents/mL) in whole blood and plasma of rats following a single oral dose of [^{14}C]BX-112 at 50 or 500 mg/kg. [a]

Time (hour)	Concentration of radioactivity (µg equivalents/mL)							
	Males				Females			
	Low-dose		High-dose		Low-dose		High-dose	
	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma
0.08	6.67	11.6 [b]	16.9	29.8	10.5	19.3	10.2	17.7
0.17	27.0	47.2	31.5	50.9 [b]	26.8	47.7	20.3	34.5 [b]
0.3	35.1	59.1	49.6	88.5 [b]	34.9	62.7	27.9	54.1 [b]
0.5	40.5 [b]	75.3 [c]	61.1	110	44.3	78.6	34.5	57.6 [b]
1	16.7	27.1 [b]	39.9	67.5	30.1	49.4	20.6 [b]	35.2 [b]
2	3.97	5.79	22.3	34.6	10.4	16.7	23.3 [c]	39.9 [c]
3	2.23 [b]	3.62 [d]	10.5	20.4	2.99 [c]	7.13 [d]	16.6 [b]	21.9 [b]
4	1.72	2.65	7.67	11.8	3.28 [b]	7.07 [d]	9.86	12.8
6	1.34 [b]	1.94 [b]	2.23	3.63	2.60 [b]	2.61 [c]	1.66	2.63
8	1.73 [b]	2.38 [b]	3.25	4.63	1.26 [b]	1.66 [b]	1.48	2.27
12	0.506 [b]	0.623 [b]	3.48	4.17	0.670 [b]	0.839 [b]	4.86	6.69
24	0.294 [b]	0.380 [b]	14.5	1.85	1.53 [b]	2.25 [b]	2.26	4.16
48	0.117 [b]	0.194 [b]	1.05	0.550	0.145	0.213	2.68	3.64
96	0.034 [b]	0.106 [b]	ND	ND	ND	0.065	1.06	1.50

a Data were obtained from Tables 4 and 5, pages 93 and 94 of MRID 44457770, and are the average of 5 rats/sex/dose group, unless otherwise indicated.
b Values are average of four animals.
c Values are average of three animals.
d Values are average of two animals.

Kinetic parameters calculated for single low- and high-dose animals are presented in Table 8. In the low-dose group, there was no difference between sexes in the absorption coefficient (K_a) and time to the inflexion. Although not statistically significant, the average half-lives for distribution (male, 0.25 hours; females, 0.37 hours) and elimination (male, 6.35 hours; females, 7.27 hours) of radioactivity in the plasma were somewhat longer in females than in males, and the AUC was also higher for females (130.9 µg eq/mL·h) than males (90.98 µg eq/mL·h). In the high-dose group, a high degree of

variability was noted in parameters between animals of the same sex, and values for several animals were not included as they were considered not physiologically acceptable. There was no difference between males and females in the absorption coefficient, distribution and elimination half-lives, and the time to the inflexion. The AUC value for plasma was higher for males (236.7 µg eq/mL·h) than females (162.6 µg eq/mL·h). However, given the limited amount of data available, comparisons of high-dose males to females are equivocal.

Comparing plasma kinetic parameters for low- and high-dose males, the absorption K_a, elimination $T_{½}$, and time to the inflexion were not affected by dose, but increasing the dose increased the distribution $T_{½}$ (1.9x) and the AUC value (2.6x) for males. Except for an increase in the distribution $T_{½}$ (2.2x), none of the plasma kinetic parameters for females were substantially affected by the dose level.

The relatively minor increases (1.2-2.6x) in plasma AUC values between low- and high-dose animals supports the observation that absorption of [^{14}C]BX-112 was limited at the high-dose level.

Table 8. Pharmacokinetic parameters calculated from blood and plasma of rats dosed with [^{14}C]BX-112 at 50 or 500 mg/kg.[a]

Kinetic Parameters	Males			
	Low dose (50 mg/kg)		High dose (500 mg/kg)	
	Blood	Plasma	Blood	Plasma
Absorption K_a (h^{-1})	2.90 ± 0.71	2.93 ± 0.62	3.07 ± 1.08	2.69 ± 1.02
Distribution $T_{½}$ (h)	0.25 ± 0.06	0.25 ±0.05	0.61 ± 0.20	0.47 ± 0.25
Elimination $T_{½}$ (h)	7.42 ± 2.12	6.35 ± 1.17	49.7 ± 52.4 [b]	9.28 ± 12.1
AUC (µg eq/mL·h)	59.31 ± 12.06	90.98 ± 15.54	254.1 ± 73.07 [b]	236.7 ± 146.2
Inflexion (h)	3.14 ± 0.88	3.44 ± 0.89	3.84 ± 0.42	3.64 ± 0.82
Kinetic Parameters	Females			
	Low dose (50 mg/kg)		High dose (500 mg/kg)	
	Blood	Plasma	Blood	Plasma
Absorption K_a (h^{-1})	2.60 ± 0.98	2.95 ± 1.44	4.37 ± 1.66	4.06 ± 0.91
Distribution $T_{½}$ (h)	0.37 ± 0.11	0.37 ± 0.12	0.64 ± 0.56	0.81 ± 0.45
Elimination $T_{½}$ (h)	10.3 ± 4.00	7.27 ± 5.16	12.1 [c]	7.44 [c]
AUC (µg eq/mL·h)	84.64 ± 30.48	130.9 ± 44.64	118.7 [c]	162.6 [c]
Inflexion (h)	3.60 ±1.25	3.56 ± 1.43	3.63 ± 3.29	4.44 ± 2.60

a Data were obtained from Tables 6-9, pages 95-98 of MRID 44457770. For the low-dose group, data are the average ± S.D. of 4 rats/sex: data were excluded for one male (27M) that died between 4-6 hours post-dose and

one female (30F) which for which blood samples were not available from 3-24 hours post-dose. For the high-dose group, data are the average ± S.D. of 5 rats/sex, unless otherwise indicated.

b Values are based on data from four males, as one male (35M) had an anomalous elimination $T_{½}$ of 201.2 hours.

c Average of two females. The report stated that data from the other three females were excluded as values were not acceptable on a physiological basis; elimination $T_{½}$s for whole blood were $>10^{25}$ hours for two females and the elimination $T_{½}$ for plasma was 85.5 hours another female.

3. Tissue distribution over time

The concentration of radioactivity in tissue, organ and blood samples from low- and high-dose rats over time are presented in Tables 9 and 10, respectively. With a few exceptions, concentrations of radioactivity in blood and tissues within each dose group were similar between sexes over time, although levels of radioactivity generally declined more gradually in tissues of females than males. Maximum concentrations of radioactivity in tissues were attained within 0.5-3 hours of dosing for the low-dose group and within 0.5-6 hours of dosing for the high-dose group. By 168 hours post-dose, average concentrations of radioactivity were ND-0.105 µg/g in the low-dose and ND-1.65 µg/g in the high dose group. There was no evidence of accumulation in specific organs or tissues.

The relative distribution of radioactivity among tissues was similar for low- and high-dose groups and between sexes. Excluding the G.I. tract, maximum concentrations of radioactivity attained in tissues/organs were highest in the lymph nodes (low-dose, 142-174 µg/g; high-dose, 190-445 µg/g), kidneys (low-dose, 96.8-99.5 µg/g; high-dose, 175-191 µg/g), pancreas (low-dose, 54.2-86.2 µg/g; high-dose, 174-179 µg/g), spleen (low-dose, 33.9-83.2 µg/g; high-dose, 87.7-91.4 µg/g), and liver (low-dose, 29.9-34.6 µg/g; high-dose, 53.6-80.6 µg/g). Relatively high levels of radioactivity were also observed in the uterus (low-dose, 76.7 µg/g; high-dose, 173 µg/g) and ovaries (low-dose, 83.6 µg/g; high-dose, 83.8 µg/g) of females. The lowest concentrations of radioactivity were initially (0.5 hour) observed in bone (low-dose, 2.87-3.27 µg/g; high-dose, 4.85-6.36 µg/g).

Increasing the dose level increased the concentration of radioactivity in tissues, but not in proportion to the 10x increase in dose level. Compared to the low-dose group, maximum concentrations of radioactivity in the above tissues were 1.8-3.3x higher in high-dose males and 1-2x higher in high-dose females.

Repeated dosing at 50 mg/kg for 14 days prior to dosing with [^{14}C]BX-112 at 50 mg/kg had no effect on the accumulation of radioactivity in tissues/organs.

a) Single low dose: With the exception of the caecum, large intestines, uterus, and muscle (females only), maximum levels of radioactivity occurred in rat tissues/organs and blood sampled at 0.5 hours. Levels of radioactivity increased in the caecum, uterus, and muscles (females only) from 0.5 to 3 hours and then declined thereafter, and radioactivity in the large intestines increased from 0.5 to 6 hours, declining thereafter. Concentrations of radioactivity in blood and tissues over time were generally similar between males and females. At 0.5 hours post-dose, concentrations of radioactivity in tissues/blood (excluding the G.I. tract) were highest in the lymph nodes (142-174 µg/g), kidneys (96.8-99.5 µg/g), pancreas (54.2-86.2 µg/g), spleen (33.9-83.2 µg/g), adrenals (36.6-52.9 µg/g), and liver (29.9-34.6 µg/g) of both males and females. In addition, females had relatively high levels of radioactivity in the uterus (58.7 µg/g) and ovaries (83.6 µg/g) at 0.5 hours post-dose. The lowest levels

of radioactivity were found in the brain (1.92-2.16 µg/g), bone (2.87-3.27 µg/g), and testes (5.64 µg/g). By 168 hours post-dose, average ^{14}C-residues (excluding G.I. tract) were present at ≥ 0.01 µg/g only in kidneys (0.026-0.036 µg/g), skin (0.058-0.073 µg/g), and bone (0.024-0.062 µg/g) of both sexes, in the plasma (0.016 µg/g) of males, and in the uterus (0.010 µg/g),) and lymph nodes (0.011 µg/g) of females.

Table 9. Distribution of radioactivity in blood, tissues, and organs of rats sacrificed at various intervals following a single oral dose of [^{14}C]BX-112 at 50 mg/kg. [a]

Interval	Concentration of radioactivity (µg equivalents/g) [b]				
	0.5 hr	3 hr	6 hr	96 hr	168 hr
Tissue/organ	Males				
Stomach	270	20.5	7.06	0.089	0.024
S. Intestine	639	88.3	33.2	0.162	0.026
Caecum	82.4	538	456	0.664	0.081
L. Intestine	56.0	84.9	119	0.576	0.105
Liver	29.9	4.20	2.69	0.039	0.009
Kidney	99.5	17.9	10.0	0.096	0.026
Lung	16.1	1.83	1.15	ND [c]	ND
Pancreas	54.2	28.9	31.1	0.112	0.007
Spleen	33.9	24.1	16.1	0.015	ND
Heart	10.1	2.24	1.06	ND	0.006
Brain	2.16	0.646	0.248	ND	ND
Testes	5.64	0.813	1.13	ND	ND
Muscle	18.5	2.87	1.54	0.015	ND
Fat	29.9	15.10	1.11	0.060	ND
Skin	20.5	7.08	2.72	0.270	0.073
Carcass	15.7	5.34	5.09	0.029	0.005
Bone	2.87	1.10	1.11	0.190	0.062
Adrenals	36.6	4.10	2.78	ND	ND
Thyroid	21.3	0.940	0.433	ND	ND
Eyes	6.73	0.745	0.345	0.014	ND
Harderian	7.27	0.686	0.451	0.005	ND
Lymph Nodes	142	84.6	62.8	0.210	ND
Salivary gland	7.67	0.433	0.637	ND	ND
Submaxillary	8.74	0.566	0.487	0.008	0.001
Thymus	7.11	0.451	0.301	0.010	ND
Blood	17.7	0.759	0.441	0.036 [d]	ND
Plasma	NS	NS	NS	0.053	0.016

(continued)

Table 9. Continued.

Interval	Concentration of radioactivity (µg equivalents/g) [b]				
	0.5 hr	3 hr	6 hr	96 hr	168 hr
Tissue/organ	Females				
Stomach	322	27.2	8.53	0.275	0.006
S. Intestine	505	90.0	43.6	0.405	0.018
Caecum	50.6	792	428	2.08	0.052
L. Intestine	58.1	67.0	154	1.93	0.049
Liver	34.6	7.73	3.09	0.064	0.003
Kidney	96.8	13.3	6.76	0.176	0.036
Lung	18.3	1.74	1.03	0.009	ND
Pancreas	86.2	13.0	19.6	0.264	ND
Spleen	83.2	14.1	6.89	0.041	ND
Heart	11.9	1.99	1.12	0.005	ND
Brain	1.92	0.551	0.301	ND	0.004
Ovaries	83.6	47.9	47.5	0.581	ND
Uterus	58.7	76.7	50.4	0.761	0.010
Muscle	8.70	11.8	4.12	0.078	ND
Fat	15.5	7.84	6.96	0.078	ND
Skin	17.9	4.83	5.18	1.92	0.058
Carcass	13.8	7.42	5.39	0.136	ND
Bone	3.27	1.10	0.758	0.042	0.024
Adrenals	52.9	7.89	2.03	0.040	ND
Thyroid	13.8	1.22	0.911	ND	ND
Eyes	9.55	0.860	0.789	0.031	ND
Harderian	9.10	1.04	0.651	0.033	ND
Lymph Nodes	174	131	112	0.696	0.011
Salivary gland	11.9	0.775	0.593	ND	ND
Submaxillary	9.99	0.635	0.624	0.015	ND
Thymus	8.48	0.509	0.321	0.017	ND
Blood	21.1	0.684	0.431	ND	ND
Plasma	NS	NS	NS	0.091	ND

[a] Data are the average of 5 animals/sex/interval, unless otherwise indicated, and were obtained from Tables 10-14, pages 99-103 of MRID 44457770.

[b] Data for whole blood and plasma are expressed as µg equivalent/mL.

[c] ND = not detected above background.

[d] Value for whole blood from one rat; level of radioactivity in blood was ND for the other three male rats.

b) Single high dose: In males, maximum concentrations of radioactivity in tissues/blood occurred within 0.5 hours of dosing with the following exceptions: caecum, large intestines, liver, lungs, spleen, heart, brain, testes, and lymph nodes. Maximum concentrations of radioactivity were attained at 3 hours post-dose in the caecum (19,800 µg/g), liver (80.6 µg/g), lungs (44.0 µg/g), spleen (87.7 µg/g), brain (10.8 µg/g), testes (20.3 µg/g), and lymph nodes (445 µg/g), and at 6 hours post-dose in the large intestines (7,690 µg/g) and heart (50.7 µg/g). The lowest relative concentrations of radioactivity in males at 0.5 hours post-dose were found in the bone and brain (6.36-6.38 µg/g). By 168 hours post-dose, radioactivity in males was <0.05 µg/g in tissues/blood, with the following exceptions: G.I. tract (0.035-0.170 µg/g), skin (1.02 µg/g), bone (1.65 µg/g), adrenals (0.165 µg/g), thyroid (0.737 µg/g), lymph nodes (0.111 µg/g), salivary glands (0.127 µg/g), and harderian and submaxillary glands (0.050 µg/g).

In females, maximum concentrations of radioactivity also occurred in most tissues/blood within 0.5 hours of dosing with the following exceptions: caecum, large intestines, liver, lungs, pancreas, spleen, heart, ovaries, uterus, carcass, harderian gland, and lymph nodes. Maximum concentrations of radioactivity were attained at 3 hours post-dose in the caecum (13,500 µg/g), liver (53.6 µg/g), pancreas (174 µg/g), heart (46.4 µg/g), ovaries (83.8 µg/g), and lymph nodes (190 µg/g), and at 6 hours post-dose in the large intestines (10,900 µg/g), lungs (74.6 µg/g), spleen (91.4 µg/g), uterus (173 µg/g), carcass (16.2 µg/g) and harderian gland (14.7 µg/g). The lowest relative concentrations of radioactivity in females at 0.5 hours post-dose were found in the bone (4.85 µg/g), and brain and muscle (7.80-7.91 µg/g). By 168 hours post-dose, radioactivity was <0.05 µg/g in tissues/blood, with the exceptions of the G.I. tract (0.037-0.104 µg/g), heart (0.081 µg/g), ovaries (0.154 µg/g), skin (0.255 µg/g), thyroid (0.734 µg/g), and lymph nodes (0.123 µg/g).

With a few exceptions, concentrations of radioactivity in blood and tissues over time were generally similar between high-dose males and females, although levels of radioactivity generally declined more gradually in tissues of females than males. In both sexes, maximum concentrations of radioactivity in tissues (excluding the G.I. tract) were highest in the lymph nodes (190-445 µg/g), kidneys (175-191 µg/g), pancreas (174-179 µg/g), spleen (87.7-91.4 µg/g), and liver (53.6-80.6 µg/g) and lowest in bone (4.85-6.36 µg/g). Relatively high levels of radioactivity were also observed in the thyroid (357 µg/g) of males, though only at the 0.5 hour interval, and in the uterus (173 µg/g) and ovaries (83.8 µg/g) of females. The most notable difference between the sexes was that males had higher levels of radioactivity at 0.5 hours post-dose in muscle (3.3x), fat (5x), thyroid (14x), pancreas (2x), and lymph nodes (2.2x) than females.

The relative distribution of radioactivity among tissues was similar for low- and high-dose groups. For animals in both dose groups, maximum residues in tissues (excluding the G.I. tract) were observed in lymph nodes, kidneys, pancreas, spleen,

liver, uterus and ovaries. Increasing the dose level increased the concentration of radioactivity in tissues, but not in proportion to the 10x increase in dose level. Compared to the low-dose group, maximum concentrations of radioactivity in these tissues were 1.8-3.3x higher in high-dose males and 1-2x higher in high-dose females.

Table 10. Distribution of radioactivity in blood, tissues, and organs of rats sacrificed at various intervals following a single oral dose of [^{14}C]BX-112 at 500 mg/kg. [a]

Interval	Concentration of radioactivity (µg equivalents/g) [b]				
	0.5 hr	3 hr	6 hr	96 hr	168 hr
Tissue/organ	Males				
Stomach	2,990	468	66.1	85.9	0.069
S. Intestine	10,200	998	128	1.24	0.035
Caecum	140	19,800	11,100	3.25	0.144
L. Intestine	161	4, 610	7,690	604	0.170
Liver	46.5	80.6	61.3	0.107	ND [c]
Kidney	175	62.5	23.5	0.323	ND
Lung	34.3	44.0	32.3	ND	ND
Pancreas	179	108	87.8	0.433	ND
Spleen	55.8	87.7	40.3	0.089	ND
Heart	32.0	33.1	50.7	ND	ND
Brain	6.38	10.8	4.51	ND	ND
Testes	13.9	20.3	8.84	0.100	ND
Muscle	25.6	16.3	2.04	ND	ND
Fat	75.5	22.1	13.0	ND	ND
Skin	25.0	5.21	5.73	0.471	1.02
Carcass	28.9	13.7	21.5	ND	0.036
Bone	6.36	3.53	0.971	0.165	1.65
Adrenals	38.3	35.8	11.7	0.179	0.165
Thyroid	357	3.90	1.89	0.866	0.737
Eyes	9.17	2.47	1.55	0.036	0.032
Harderian	13.3	2.75	2.37	0.150	0.054
Lymph Nodes	178	445	238	1.16	0.111
Salivary gland	17.8	3.27	2.40	0.303	0.127
Submaxillary	17.2	2.24	1.13	0.053	0.050
Thymus	10.6	2.68	1.19	0.050	ND
Blood	28.8	4.03	1.07	ND	ND
Plasma	48.1	7.04	1.88	ND	ND

(continued)

Table 10. Continued.

Interval	Concentration of radioactivity (µg equivalents/g) [b]				
	0.5 hr	3 hr	6 hr	96 hr	168 hr
Tissue/organ	Females				
Stomach	5,070	292	29.1	5.43	0.097
S. Intestine	6,060	1,220	155	2.99	0.037
Caecum	90.0	13,500	12,000	8.61	0.082
L. Intestine	97.0	9,750	10,900	8.90	0.104
Liver	44.9	53.6	44.2	0.386	ND
Kidney	191	76.7	63.3	2.22	ND
Lung	31.4	34.5	74.6	2.73	ND
Pancreas	87.1	174	119	6.84	ND
Spleen	60.2	52.4	91.4	5.01	ND
Heart	28.4	46.4	42.7	2.47	0.081
Brain	7.91	3.04	5.89	0.835	ND
Ovaries	75.1	83.8	63.9	0.936	0.154
Uterus	69.1	117	173	26.1	ND
Muscle	7.80	6.62	5.49	ND	ND
Fat	15.2	15.4	12.6	ND	ND
Skin	23.8	6.73	13.9	3.37	0.255
Carcass	13.3	9.76	16.2	0.033	0.032
Bone	4.85	2.45	ND	ND	ND
Adrenals	27.5	12.2	6.41	0.216	ND
Thyroid	25.2	5.01	2.24	ND	0.734
Eyes	7.84	3.50	4.62	0.048	0.021
Harderian	13.4	9.75	14.7	ND	ND
Lymph Nodes	79.2	190	167	1.38	0.123
Salivary gland	15.7	9.92	2.16	ND	ND
Submaxillary	13.0	3.64	0.981	0.017	0.028
Thymus	9.14	2.90	0.717	0.051	ND
Blood	29.7	8.67	1.67	ND	ND
Plasma	51.5	15.0	3.01	0.078	0.025

[a] Data are the average of 5 animals/sex/interval, unless otherwise indicated, and were obtained from Tables 15-19, pages 104-108 of MRID 44457770.

[b] Data for whole blood and plasma are expressed as µg equivalent/mL.

[c] ND = not detected above background.

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c) Repeated low dose: The concentrations of radioactivity in tissues of rats 168 hours after a dose of [^{14}C]BX-112 at 50 mg/kg following a 14-day pretreatment with BX-112 at 50 mg/kg/day are presented in Table 11; data for tissues from single low-dose rats at 168 hours post-dose are also included for comparison. As with the single low-dose animals, radioactivity in tissues of repeated low-dose rats were low at 168 hour post-dose. Excluding the G.I. tract, average ^{14}C-residues in repeated-dose rats were <0.01 µg/g in all tissues except: kidneys (0.015-0.042 µg/g), pancreas (0.008-0.011 µg/g), skin (0.040-0.079 µg/g), carcass (0.008-0.012 µg/g), lymph nodes (0.049-0.088 µg/g), ovaries (0.035 µg/g), uterus (0.061 µg/g), muscle (0.059 µg/g, females only), adrenals (0.016 µg/g, males only), and thyroids (0.077 µg/g, males only). Compared to the single low-dose group, radioactivity was slightly higher in the thyroid and lymph nodes of repeated low-dose males and in the ovaries, uterus, muscle, and lymph nodes of repeated low-dose females. However, accumulation of radioactivity in tissues was not evident.

Table 11. Distribution of radioactivity in blood, tissues, and organs of rats sacrificed 168 hours following a single or repeated oral dose of BX-112 at 50 mg/kg. [a]

	Concentration of radioactivity (µg equivalents/g) [b]			
	Single low-dose		Repeated low-dose	
Tissue/organ	Males	Females	Males	Females
Stomach	0.024	0.006	0.006	ND [c]
S. Intestine	0.026	0.018	0.042	0.032
Caecum	0.081	0.052	0.081	0.113
L. Intestine	0.105	0.049	0.096	0.087
Liver	0.009	0.003	ND	ND
Kidney	0.026	0.036	0.015	0.042
Lung	ND	ND	ND	ND
Pancreas	0.007	ND	0.011	0.008
Spleen	ND	ND	ND	ND
Heart	0.006	ND	ND	ND
Brain	ND	0.004	ND	ND
Testes	ND	--	ND	--
Ovaries	--	ND	--	0.035
Uterus	--	0.010	--	0.061
Muscle	ND	ND	ND	0.059
Fat	ND	ND	ND	ND
Skin	0.073	0.058	0.040	0.079
Carcass	0.005	ND	0.012	0.008
Bone	0.062	0.024	ND	ND
Adrenals	ND	ND	0.016	ND
Thyroid	ND	ND	0.077	ND
Eyes	ND	ND	0.004	0.007
Harderian	ND	ND	ND	ND
Lymph Nodes	ND	0.011	0.049	0.088
Salivary gland	ND	ND	ND	0.011
Submaxillary	0.001	ND	0.002	0.002
Thymus	ND	ND	ND	0.003
Blood	ND	ND	ND	ND
Plasma	0.016	ND	ND	ND

[a] Data are the average of 5 animals/sex/dose group and were obtained from Tables 14 and 21, pages 103 and 110 of MRID 44457770.

[b] Data for whole blood and plasma are expressed as µg equivalent/mL.

[c] ND = not detected above background.

4. Excretion

The recovery of radioactivity in excreta of male and female rats from all dose groups in the mass balance study is presented in Table 12. With the exception of bile-cannulated rats, which were terminated 24 hours after dosing, 91.8-109% of the dosed radioactivity was recovered from males and females within 168 hours of oral dosing with [^{14}C]BX-112 at 50 or 500 mg/kg. There were no sex-related differences in the pattern of excretion, and pretreatment had no effect on the amount or route of excretion. For males and females dosed orally with [^{14}C]BX-112 at 50 mg/kg (with or without pretreatment) renal excretion was the primary route of elimination accounting for 76.2-84.6% of the dose; fecal excretion accounted for 17.2-24.7% of the dose. Biliary excretion of radioactivity was minor, accounting for ≤0.3% of the dose in bile-cannulated rats. When the dose was increased to 500 mg/kg, both males and females eliminated the majority of the dose in the feces (58.2-60.9% dose), with renal excretion accounting for 33.6-40.1% of the dose. With the exception of bile-cannulated rats, which were sacrificed within 24 hours of dosing, ≤0.1% of the dose remained in the carcass of both sexes by 168 hours post-dose.

Table 12. Recovery of radioactivity in tissues/carcass and excreta of rats dosed orally with [^{14}C]BX-112 at 50 or 500 mg/kg.[a]

Dose	Percent of radioactive dose administered							
	Single low dose (50 mg/kg)		Single high dose (500 mg/kg)		Repeated low dose (50 mg/kg)		Single low dose - bile cannulated (50 mg/kg)	
Sample	Male	Female	Male	Female	Male	Female	Male	Female
Urine	58.3	54.5	16.0	9.5	64.4	50.0	57.8	31.8
Cage washes	24.4	21.7	24.1	24.1	14.7	34.6	26.2	30.6
Feces	17.2	20.0	60.9	58.2	23.6	24.7	10.8	2.7
Bile	NA[b]	NA	NA	NA	NA	NA	0.2	0.3
Tissues/Carcass	0.1	<0.05	<0.05	<0.05	<0.05	<0.05	NA	NA
GI tract/liver	NA	NA	NA	NA	NA	NA	2.3	11.3
Total	100	96.2	101	91.8	103	109	97.3	76.7

a With the exception of the bile-cannulated rats, data are the mean of 5 rats/sex/dose group and were obtained from Tables 2, 3, and 20 (pages 91, 92, and 109 of MRID 44457770). Data for the low-dose bile-cannulated rats are the mean of 4 rats/sex and were obtained from Table 22 (page 111 MRID 44457770); bile-cannulated rats were terminated 24 hours post-dosing.

b NA = not applicable.

C. Metabolite Characterization

Quantitative RP-HPLC (C_{18}) analyses isolated up to 4 and 3 distinct radioactive components in urine and fecal extracts, respectively. The results of these analyses are summarized in Table 13. The proposed pathway for biotransformation of BX-112 in rats is presented as an attachment.

All metabolite fractions accounting for ≥5% of the dose in extracts of urine and feces were characterized and/or identified. The principle metabolite fraction detected in extracts of both urine and feces was identified by LC/MS as the free acid metabolite of BX-112 (KI-2817). Minor amounts (≤2.3% dose) of the despropionyl free acid of BX-112 (KI-5376) were also identified by HPLC cochromatography in fecal extracts of high-dose animals and repeated dose-males.

A major (>10% dose) unknown metabolite, which was more polar than KI-2817, was also isolated from urine of each dose group. Enzymatic and acid/base hydrolyses of urine indicated that this unknown was unaffected by treatment with β-glucuronidase (with and without sulfatase activity) and mild acid (0.2 N HCl), but was at least partly converted to KI-2817 by base hydrolysis (1M NaOH). Initial LC/MS analysis of this unknown also indicated that KI-2817 was a component of the molecule. The study authors postulated that the unknown component was an ester conjugate of KI-2817 with β-glucuronic acid that had undergone acyl migration to form a rearranged glucuronide conjugate. However, in a subsequent report (MRID 44457773), FAB/MS and LC/MS analyses failed to provide conclusive evidence that the compound was a glucuronide conjugate, although the analyses did confirm that the compound could be degraded to KI-2817.

Including the postulated, base-labile conjugate of KI-2817, characterized in urine, analyses of urine and fecal extracts identified and/or characterized 55.8-75.3% of the dosed radioactivity for each dose group. Metabolism of BX-112 was qualitatively and quantitatively similar between sexes, although there were minor quantitative differences between males and females in the high-dose and repeated low-dose groups. Metabolism was also qualitatively similar between dose groups.

In the low-dose group, the major metabolite in excreta was KI-2817 (38.3-39.1% dose), which was excreted primarily in the urine (20.2-25.0% dose). The only other significant component isolated from excreta of low-dose rats was the putative base-labile conjugate of KI-2817, which accounted for 17.5-17.7% of the dose in urine. In the high-dose group, KI-2817 (60.9-68.0% dose) was also the major metabolite in excreta. However, the majority of KI-2817 (53.4-64.6% dose) from high-dose rats was recovered in the feces rather than in urine, and levels of both KI-2817 and its putative base-labile conjugate were lower (3.7-6.6% dose) in urine. Minor amounts of KI-5376 (1.1-2.3% dose) were also identified in the feces of high-dose rats. Repeated dosing with BX-112 at 50 mg/kg/day, had only a minor effect on the metabolism of BX-112, slightly increasing the levels of KI-2817 in urine and feces of both sexes and its putative base-labile conjugate in urine of males. As in the single low-dose

group. KI-2817 (44.2-53.7% dose) was the principal metabolite in excreta of repeated low-dose rats, with the majority being recovered in the urine (22.9-31.6% dose), and the putative base-labile conjugate of KI-2817 accounted for 15.5-21.3% of the dose in urine.

Table 13. Metabolite profile in excreta from rats dosed with [^{14}C]BX-112.

Dose Group	Percent of administered dose					
	Single low dose (50 mg/kg)		Single high dose (500 mg/kg)		Repeated low dose (50 mg/kg)	
Metabolite/fraction[a]	Male	Female	Male	Female	Male	Female
Identified Urinary Metabolites [a]						
KI-2817	25.0	20.2	7.5	3.4	31.6	22.9
Identified Fecal Metabolites [a]						
KI-2817	14.1	18.1	53.4	64.6	22.1	21.3
KI-5376	--[b]	--	1.1	2.3	0.3	--
Total identified Metabolites	**39.1**	**38.3**	**62.0**	**70.3**	**54.0**	**44.2**
Unknown Urinary Metabolites [a]						
Unknown 3	--	0.6	--	--	0.8	--
KI-2817-conjugate 1 [c]	17.7	17.5	6.6	3.7	21.3	15.5
KI-2817-conjugate 2	2.7	4.2	0.7	--	1.9	2.2
HPLC Residual [d]	6.8	5.1	1.3	1.4	5.9	5.0
Unknown Fecal Metabolites [a]						
Unknown 2	--	--	0.7	--	--	0.7
Unknown 6	--	--	--	--	0.6	--
HPLC Residual [d]	1.7	0.7	0.9	1.1	0.4	2.4
Total Isolated Unknowns	**28.9**	**28.1**	**10.2**	**6.2**	**30.9**	**25.8**
Unanalyzed Fractions						
Residual urine (0-48 hr) [e]	1.4	1.6	0.6	0.3	2.6	1.6
Urine (48-168 hr) [f]	0.9	0.6	0.3	0.1	0.7	0.9
Residual fecal solids (0-48 hr) [e]	3.7	4.1	8.3	9.2	6.0	5.0
Feces (48-168 hr) [f]	0.6	0.5	0.1	0.4	0.7	0.4
Cage wash (0-168 hr) [f]	24.3	21.6	23.9	24.0	14.8	34.6
Total accounted for [g]	**98.9**	**94.8**	**105.4**	**110.5**	**109.7**	**112.5**

a Data are from RP-HPLC (C_{18} column) analyses of pooled urine and fecal extracts (0-48 hour) from 5 rats/sex/dose group and were obtained from Table 1, page 46 of MRID 44457771.

b -- = Not detected.

c Putative base-labile conjugate of KI-2817.

d Residual radioactivity collected from HPLC analyses that was not associated with any distinct peak.

e Data represent the unextracted radioactivity from 0-48 hour urine and fecal samples and were obtained from Table 1, page 46 of MRID 44457771.

f Data were obtained from Tables 2-4 of this review.

g Total accounted for = (Total identified) + (Total isolated unknowns) + (Total unanalyzed).

In addition to identifying KI-2817 as the major metabolite in excreta, RP-HPLC analyses of liver and kidney extracts from tissues sampled at 0.5 hour post-dose also identified KI-2817 as a major component in these tissues. In one low-dose male, KI-2817 accounted for 81.2% and 70.1% of the radioactivity extracted from liver and kidney, respectively (28.5% and 58.5% of the total tissue radioactivity). Two other minor unknowns, each at <20% of the extracted radioactivity were also detected in liver and kidney of the low-dose male. In a high-dose male, KI-2817 was the only component detected in extracts of liver and kidney, accounting for 91.8-93.1% of the extracted radioactivity, or 55.3% and 76.6% of the total radioactivity in kidney and liver, respectively.

II. DISCUSSION

A. Investigator's Conclusions

The study authors concluded that BX-112 was rapidly absorbed from the G.I. tract of male and female rats following oral dosing. In both the low- and high-dose groups, maximum concentrations of radioactivity in blood and most tissue/organs were attained within 0.5 hour of dosing and declined thereafter. By 168 hours post-dose, ≤0.1% of the dosed radioactivity remained in the body of rats from each dose group. Excretion of radioactivity was rapid and occurred mainly via the urine in the low-dose groups and via the feces in the high-dose group, with biliary excretion accounting for <1% of the dose. The pattern and rate of excretion were similar between sexes, and pretreatment did not affect the rate or route of excretion. There was also no evidence of accumulation of radioactive material after repeated dosing. The higher rates of excretion in the feces and the lower than excepted concentrations of radioactivity in the blood and tissues of high-dose rats indicate that absorption was reduced at the increased dose level.

In each dose group, radioactivity was mostly unmetabolized and excreted as the free acid, KI-2817. The only other major metabolite (>5% dose), which was isolated from urine, was characterized as a base-labile conjugate of KI-2817. This metabolite was postulated to be a glucuronide conjugate of KI-2817 that had undergone acyl migration to form a rearranged glucuronide. However, its structure could not be confirmed by FAB/MS or LC/MS analyses, although the analyses confirmed the presence of a compound which could be degraded to KI-2817.

B. Reviewer's Discussion

Absorption of [^{14}C]BX-112 from the G.I. tract of rats was evident in both low- and high-dose rats based upon the renal excretion of radioactivity. There were no sex-related differences in the overall absorption of radioactivity for both low- and high-dose animals, and pretreatment had no effect on absorption. However, absorption was reduced relatively at the high-dose level. Cumulative renal excretion (including cage wash) accounted for 76.2-84.6% of the dose for low-dose animals and 33.6-40.1% of the dose for high-dose animals.

Following oral dosing with [^{14}C]BX-112 at 50 or 500 mg/kg, 91.8-109% of the dosed radioactivity was recovered from male and female rats within 168 hours. There were no sex-related differences in the pattern of excretion, and pretreatment had no effect on the amount or route of excretion. For low-dose animals (with or without pretreatment), renal excretion was the primary route of elimination accounting for 76.2-84.6% of the dose, with fecal excretion accounting for 17.2-24.7% of the dose. Biliary excretion of radioactivity was minor, accounting for ≤0.3% of the dose in bile-cannulated rats. When the dose was increased to 500 mg/kg, fecal excretion (58.2-60.9% dose) became the primary route of elimination, with renal excretion decreasing to 33.6-40.1% of the dose. By 168 hours post-dose, ≤0.1% of the dose remained in the carcass for each dose group. Data from the preliminary study also indicated that expired organic volatiles and CO_2 each accounted for ≤0.1% of the dose for both low- and high-dose animals.

In both the low- and high-dose groups, the average concentration of radioactivity in both whole blood and plasma increased to maximum levels by 0.5 hours post-dose and declined thereafter. In males, the absorption K_a, elimination $T_{½}$, and time to the inflexion were not affected by dose, but increasing the dose increased the distribution $T_{½}$ (1.9x) and the AUC value (2.6x). Except for an increase in the distribution $T_{½}$ (2.2x), none of the plasma kinetic parameters for females were substantially affected by the dose level. The relatively minor increases (1.2-2.6x) in plasma AUC values between low- and high-dose animals supports the observation that absorption of [^{14}C]BX-112 was limited at the high-dose level.

Concentrations of radioactivity in most tissues/organs within each dose group were similar between the sexes over time, although levels of radioactivity generally declined more gradually in tissues of females than males. Maximum concentrations of radioactivity in tissues were attained within 0.5-3 hours of dosing for the low-dose group and within 0.5-6 hours of dosing for the high-dose group. By 168 hours post-dose, average concentrations of radioactivity were ND-0.105 µg/g in the low-dose and ND-1.65 µg/g in the high dose group. There was no evidence of accumulation in specific organs or tissues.

The relative distribution of radioactivity among tissues was similar for low- and high-dose groups and between sexes. Excluding the G.I. tract, maximum concentrations of radioactivity attained in tissues/organs were highest in the lymph nodes (142-445 µg/g), kidneys (96.8-191 µg/g), pancreas (54.2-179 µg/g), spleen (33.9-91.4 µg/g), and liver (29.9-80.6 µg/g). Relatively high levels of radioactivity were also observed in the uterus (76.7-173 µg/g) and ovaries (83.6-83.8 µg/g) of females. The lowest concentrations of radioactivity were initially (0.5 hour) observed in bone (2.87-6.36 µg/g). Increasing the dose level increased the concentration of radioactivity in tissues, but not in proportion to the 10x increase in dose level. Compared to the low-dose group, maximum concentrations of radioactivity in the above tissues were 1.8-3.3x higher in high-dose males and 1-2x higher in high-dose females. Repeated dosing at 50 mg/kg for 14 days prior to dosing with [^{14}C]BX-112 at 50 mg/kg had no effect on the accumulation of radioactivity in tissues/organs.

Analyses of urine and fecal extracts identified and/or characterized 55.8.4-75.3% of the dosed radioactivity for each dose group. Metabolism of BX-112 was qualitatively and quantitatively similar between sexes, although there were minor quantitative differences between males and females in the high-dose and repeated low-dose groups. Metabolism was also qualitatively similar between dose groups.

In the low-dose group, the major metabolite in excreta was identified as KI-2817 (38.3-39.1% dose), which was excreted primarily in the urine (20.2-25.0% dose). The only other significant component isolated from excreta of low-dose rats was the putative base-labile conjugate of KI-2817, which accounted for 17.5-17.7% of the dose in urine. In the high-dose group, KI-2817 (60.9-68.0% dose) was also the major metabolite in excreta. However, the majority of KI-2817 (53.4-64.6% dose) from high-dose rats was recovered in the feces rather than in urine, and levels of both KI-2817 and its putative base-labile conjugate were lower (3.4-7.5% dose) in urine of high-dose animals. Minor amounts of KI-5376 (1.1-2.3% dose) were also identified in the feces of high-dose rats. Repeated dosing with BX-112 at 50 mg/kg/day, had only a minor effect on the metabolism of BX-112, slightly increasing the levels of KI-2817 in urine and feces of both sexes and its putative base-labile conjugate in urine of males. As in the single low-dose group, KI-2817 (44.2-53.7% dose) was the principal metabolite in excreta of repeated low-dose rats, with the majority being recovered in the urine (22.9-31.6% dose), and the putative base-labile conjugate of KI-2817 accounted for 15.5-21.3% of the dose in urine.

The free acid, KI-2817, was also identified as the major metabolite extracted from kidneys and liver of low and high dose males, accounting for 28.5% and 58.5% of the total radioactivity in kidney and liver of low dose males and 55.3% and 76.6% of the total radioactivity in kidney and liver of high dose males.

IV. STUDY DEFICIENCIES

A major polar unknown found in urine, accounting for 3.7-21.3% of the dose, was not conclusively identified. This unknown, which is more polar than KI-2817, was unaffected by treatment with β-glucuronidase (with and without sulfatase activity) and mild acid (0.2 N HCl), but released KI-2817 upon base hydrolysis. Preliminary LC/MS analysis suggested that the unknown was a re-arranged glucuronide ester of KI-2817. However, subsequent FAB/MS and LC/MS analyses did not support this structural assignment, but did confirm that the compound could be degraded to KI-2817. Although this unknown was not conclusively identified, sufficient data were presented characterizing the component as a base-labile "conjugate" of KI-2817.

This study is classified **acceptable (§85-1)** and satisfies the requirements for a metabolism study in rats.

DER's metabolism in Rats, MRID NOS. 44457770 thru 44457773

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